

TITLE: A Phase I/II Double-Blinded Randomized Prospective Study of Sterile Amniotic Fluid Filtrate Epidural Injection for the Treatment of Lumbosacral Radicular Pain due to Spinal Stenosis: Improving Safety and Outcomes in the Treatment of Pain and Disability Related to Spinal Stenosis.

Version 15 (03MAR2025)

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Protocol Version: **Version 15, 03MAR2025**

Study Center: **University of Utah Orthopaedic Center**

I, the undersigned, have read and understand the protocol specified above and agree on its content. I agree to perform and conduct the trial as described in the protocol and in accordance with applicable laws and regulations. In addition, when applicable, I agree to enlist sub-investigators who also agree to perform and conduct the trial as described in the protocol.

Zachary McCormick, MD

Principal Investigator Printed Name

Principal Investigator Signature

Date

1. SYNOPSIS

Title	A Phase I/II Double-Blinded Randomized Prospective Study Standard of Sterile Amniotic Fluid Filtrate Epidural Injection for the Treatment of Lumbosacral Radicular Pain due to Spinal Stenosis: Improving Safety and Outcomes in the Treatment of Pain and Disability Related to Spinal Stenosis.
Short Title	The Lumbar Amniotic Fluid Trial
IRB Number	131761
IND	IND 26950
Phase	Phase I/II
Design	A Double-blinded, randomized, prospective study to evaluate the standard of care (Corticosteriod) vs. Sterile Amniotic Fluid Filtrate Epidural Injection for the treatment of Lumbosacral Radicular Pain due to Spinal Stenosis. Participants, study investigators, and research personnel collecting data will remain blinded to participant treatment allocation.
Study Duration	Up to 27 months
Study Center(s)	University of Utah Orthopedic Center University of Utah South Jordan Health Center
Objectives	Primary Objectives: - To determine the safety of using processed Amniotic Fluid Allograft (hAF) in participants with Lumbosacral Radicular Pain due to Spinal Stenosis. - To determine the rate of adverse events associated with hAF versus dexamethasone epidural injection as assessed by a standardized survey and review of systems. - To determine the pain level as measured by an 11-point numeric pain rating scale (NPRS) following the injection of hAF relative to corticosteroids. - To compare cross-over to alternative assignment at 3 months between groups. - To compare treatment withdrawal rates through 3-months between groups. Secondary Objectives: - To evaluate the impact on opioid and non-opioid analgesic use reduction. - To evaluate functional and psychological outcomes as measured by the Oswestry Disability Index (ODI), Swiss Spinal Stenosis Questionnaire (SSSQ), Patient Reported Outcomes Measurement Information System (PROMIS) Global-10, Patient Global Impression of Change (PGIC). - To evaluate the rate of surgical sparing for the refractory spinal stenosis symptoms. - To determine the need for additional healthcare utilization and the impact on cost.

Number of Participants	61 total participants (1:1 randomization)
Inclusion Criteria	1. Age $\geq$ 18 years. 2. Pain $\geq$ 4 in unilateral or bilateral leg (with or without back pain). Radicular distribution of leg pain based on history and correlation with advancing imaging. Radicular pain may be fixed or claudicatory in nature. (Score based on Baseline Survey NPRS for leg pain.) 3. Pain resistant to a trial of conservative therapy (i.e., oral steroids, nonsteroidal anti-inflammatory drugs (NSAIDs), opioids, muscle relaxants, physical therapy, chiropractic or other non-invasive care) for at least three months. 4. Mild-moderate-severe lumbar foraminal or subarticular zone stenosis, and/or mild-moderate central canal spinal stenosis identified by magnetic resonance imaging (MRI) or computerized tomography (CT) scan according to radiologic criteria (Boden 1996). 5. Ability to read English or Spanish and complete the assessment questionnaires. 6. Must have been 90 days since last lumbar steroid injection.
Exclusion Criteria	1. Participants in the opinion of the treating investigator who are unwilling or unable to comply with study procedures. 2. Systemic infection or local infection over planned injection site. 3. Bleeding disorder, current use of anticoagulant medications. 4. Intrinsic spinal cord lesions. 5. History of central neurologic, cerebrovascular, demyelinating or muscular disease. 6. Severe vascular, pulmonary or coronary artery disease that limits ambulation including recent myocardial infarction (within the last 6-months). 7. Allergy to medications being used for injection procedures. 8. Women who are pregnant, breastfeeding, or plan to become pregnant while participating in the study. If of child-bearing potential, unwillingness to use effective birth control while participating in the study. 9. Cognitive deficit or motor neuron disease. 10. Spinal instability requiring surgery. 11. History of lumbar spinal fusion surgery 12. Metastatic cancer. 13. Concordant pain with internal rotation of the hip (or known hip joint pathology).

Study Product, Dose, Route, Regimen	Standard of care steroid injection includes dexamethasone sodium phosphate 10 mg (1mL, 10 mg/mL) combined with 2 mL of sterile water will be injected through the spinal needle into the epidural space for unilateral symptoms. The amniotic fluid injection will also be given as 3 mL of drug injected through the spinal needle into the epidural space for unilateral symptoms.
Statistical Methodology	Outcomes will be assessed at 3 weeks, 6 weeks, 3 months and 6 months for all participants. On February 7, 2025, the Data Safety Monitoring Board recommended ending the study enrollment early. This recommendation was due to the amniotic fluid group demonstrating significantly inferior patient reported outcomes related to pain reduction and a significantly higher rate of repeat injection when compared to the dexamethasone (standard care) group. Notably, the Data Safety Monitoring Board had no safety concerns, and the recommendation was not based on safety differences between the two study groups. As such, enrollment was stopped at 61 participants. 12- and 24-month data will no longer be collected for participants who already surpassed 6 months of follow-up following the Data Safety Monitoring Board's recommendation. Per the original protocol, 12- and 24-month data was already collected for participants who were enrolled and reached these follow-up milestones prior to the Data Safety Monitoring Board's recommendation. Analytic approach: Intention to treat analyses for primary effectiveness analysis will be used; this evaluates outcomes based on the treatment arm to which participants were originally assigned. Long-term outcomes past 3 months will be evaluated using both intention-to-treat and as-treated analysis given cross-over and drop-out. A superiority analysis will be conducted for all outcome measures. Adverse events from a standardized survey and review of systems will be tallied and compared between groups. Data will first be checked for distributional form and outliers using summary statistics and graphical displays. Data will be analyzed using SPSS version 22 (IBM 2013) or STATA-MP-16 (College Station, TX). The level of significance will be set at 0.05. Two-sided testing will be used for all hypothesis testing. In comparing groups with regard to categorical variables, Chi Square or Fisher's exact tests will be used. Proportions and 95% Confidence Intervals will also be calculated to confirm that any differences between groups are truly distinguished. Depending on the distribution of continuous data, Student's T-tests or Mann-Whitney U tests will be

	used to compare means or medians, respectively. Categorical measures of both pain and function will be used for the primary outcome analysis given recommendations by the National Institute of Health's low back pain research consortium. Multiple regression analysis will be used to determine if any demographic or clinical variables (age, BMI, duration of pain, etc.) are independently associated with successful pain (NRS) and functional (ODI, SSSQ, etc.) outcomes. Longitudinal models of outcome using a time-varying covariate indicating when treatment occurred will be used in order to account for changes in outcome related to group cross-over. On February 7, 2025, the Data Safety Monitoring Board recommended ending the study enrollment early. This recommendation was due to the amniotic fluid group demonstrating significantly inferior patient reported outcomes related to pain reduction and a significantly higher rate of repeat injection when compared to the dexamethasone (standard care) group. Notably, the Data Safety Monitoring Board had no safety concerns, and the recommendation was not based on safety differences between the two study groups. As such, an interim statistical analysis will be performed using the data from the reduced number of trial participants enrolled (n= 61)
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2. BACKGROUND

Lumbar spinal stenosis is a common cause of chronic pain and disability in older adults and is the leading reason for spinal surgery in older adults^{1,2}. In this condition, degenerative changes in intervertebral discs and osseous anatomy of the spine result in a narrowing of the spinal canal and neuroforamen in close proximity to the traversing spinal nerve roots, causing back and radicular leg pain, paresthesias and weakness^{3,4}. In the absence of a progressive neurologic deficit, initial treatment of lumbosacral radicular pain includes physical therapy, activity modification, and oral neuropathic or analgesic medications^{5,6}. If this conservative approach fails to relieve pain, an image-guided epidural steroid injection (ESI) is the second-line treatment^{5,6}. Surgical decompression may be a treatment option for some patients who fail conservative therapy, but it is not uniformly effective and is associated with substantial direct and indirect risks^{7,8}. Further, some patients are unwilling to undergo invasive surgical treatments, and in others, surgery is contraindicated due to medical co-morbidities.

Epidural steroid injections are widely used for the treatment of spinal stenosis. Over 25% of all epidural steroid injections in the Medicare population are administered for the treatment of pain associated with spinal stenosis, accounting for more than 500,000 injections per year^{9,10}. When performed with sterile technique and fluoroscopic guidance, epidural spine injections of steroids are safe^{11,12,13}. However, steroid use in the epidural space is associated with rare but catastrophic sequelae including paraplegia due to spinal cord infarction¹⁴⁻²¹.

The larger size or aggregation of particulate steroids likely obstructs arteriolar blood flow to the spinal cord if inadvertently injected into radicular arteries during TFESI²²⁻²⁷. In addition, epidural steroid use is associated with common immediate/short-term adverse events such as facial flushing, headache, insomnia, transient hypertension, and increased blood glucose in patients with diabetes²⁸. Injections must often be repeated when administered for chronic radicular pain given a typical natural history of life-long pain associated with this condition, which increases the risk of long-term sequelae including osteoporosis, adrenal suppression, hypertension, cataracts, gastrointestinal bleeding, and immune system dysfunction among many others²⁹.

Furthermore, the injection treatment paradigm for patients with spinal stenosis has not been optimized. While transforaminal epidural steroid injection appears to be superior to both placebo saline injection and lidocaine injection at short-term to intermediate follow-up when performed for the indication of radicular pain due to acute disc herniation³⁰, the highest quality evidence to date suggests a lesser degree of efficacy for the indication of leg pain due to spinal stenosis³¹⁻³⁴. This is particularly problematic given that radicular pain related to disc herniation is self-limited, whereas spinal-stenosis related radicular pain does not tend to improve over time^{35,36}. Thus, there is a large group of patients with spinal stenosis who fail to respond to physical therapy and oral medication management, yet either wish to avoid spinal surgery or are not eligible, and suffer from chronic pain and debility given the poor treatment options. Clearly, better treatments with more optimal safety and side-effect profiles compared to epidural corticosteroids must be investigated in this context.

There is therefore a critical need to identify safe and effective treatments for this common clinical condition, which would allow for improved patient function and pain relief. Amniotic Fluid (AF) is a promising new biologic treatment with neuroprotective and regenerative properties. Early after conception and until the mother's water breaks for the delivery of their infant, the fetus is bathed in amniotic fluid. AF functions as a supportive cushion to the fetus and provides a protective environment. AF is a rich source of nutrients, cytokines and growth factors that are required for fetal development and maturation³⁷. AF also contains multiple cell types with the potential to differentiate along multiple cell lineages^{38,39}. The protective and regenerative properties of AF are achieved via the exchange of water and solutes with surrounding tissues. This is accomplished via the utilization of different pathways during the course of a pregnancy that likely contributes to changes in the composition of AF with gestational age³⁷.

Early evidence demonstrates that concentrates of AF inhibit the development of peritonitis and accelerate defense-repair mechanisms within damaged joints⁴⁰, demonstrating protective biological properties⁴¹. Since these early publications, more sophisticated evaluations have revealed the presence of antimicrobial, immunomodulatory, and growth-promoting activities of AF³⁷. For example, low AF antimicrobial activity is associated with a high incidence of infectious syndromes in pregnant women⁴². Components with antimicrobial, antiviral and antifungal activity that are present in AF include lysozyme, peroxidase, transferrin, beta-lysin, immunoglobulins and zinc-peptide complexes⁴³. Immunomodulatory properties of AF are evident from studies showing that enteral feeding of AF suppresses the pro-inflammatory responses in preterm pigs with necrotizing enterocolitis⁴². Further, growth-promoting activities of AF are supported by both animal studies and *in vitro* studies, showing that AF can enhance neochondrogenesis⁴⁴, regenerate peripheral nerves⁴⁵ and bone⁴⁶, accelerate re-epithelialization in corneas⁴⁷, and promote healing of human skin wounds⁴⁸.

Some of the factors that are found in AF that may contribute to these activities include inflammatory mediators such as TNF- α , IL-6, IL8, and IL-10⁴⁸ trophic factors that include EGF, IGF-1, FGF, HGF and TGF- α ^{49,50}, and hyaluronic acid, an important factor in promoting re-epithelialization in human skin wounds⁴⁸.

Human AF also contains factors that appear to minimize scarring and adhesions⁴⁵. Ozgenel et al, describe how adhesions and scarring are reduced or eliminated in a peripheral nerve rat model. It is interesting that a fetal incision made early in gestation will heal without a scar whereas one made in late gestation heals with scar formation. Hyaluronic acid, which is found in high levels in AF, inhibits collagen synthesis. This hyaluronic acid-rich environment is due to a relative lack of hyaluronidase in AF and to the presence of hyaluronic acid-stimulating factor in AF⁵¹. In a study investigating the effect of AF on proteases important to wound healing, human AF was shown to enhance collagenase activity but to inhibit activities of hyaluronidase, elastase, and cathepsin⁵².

Our team has already conducted studies to assess the hypothesis that nutrients, cytokines and growth factors contained in the non-cellular fraction of AF are useful for reparative and regenerative treatments in patients⁵³. The first aim was to determine the feasibility of consenting and screening volunteer donors for the routine collection of AF from full-term pregnant women scheduled for caesarean-section (C-sections) and then processing the AF for clinical applications. The second aim was to develop a processing method that resulted in a cell-free AF preparation suitable for clinical applications. The third goal was to gain a better understanding of the components of AF procured from full-term pregnancies. Initially, 36 pregnant women consented and passed the donor screening criteria. AF was successfully collected from 17 individuals. Median AF volumes were 70 mL (range 10–815 mL; n = 17). Fluid chemistries were similar, but some differences were noted in HA levels and cytokine profiles. Cytokine arrays revealed that an average of 304 ± 20 (mean $\pm$ SD; n=3) of 400 proteins tested were present in AF with a majority of cytokines associated with host defense. The proteins examined were annotated in the protein arrays as anti-inflammatory or pro-inflammatory. Twelve (12) of 17 (70%) of proteins known to have Anti-inflammatory cytokines were detected in the AF samples, while only 5 of 14 (36%) pro-inflammatory proteins were detected in AF samples. There were no (TNF)- α , or IL-1 β found in AF samples. Three (3) cytokines were detected with both pro- and anti-inflammatory activity. Some of the peptides encountered and classified according to their function are found in **Table 1 below**:

Table 1

Pro-inflammatory	OPN, PAI-I, CD163, RAGE, IL17, IL1R3
Host defense	IL-27, LAG-3, GITR, PD1
Innate Immunity	hCGb, Galectin-3, TLR-2, Osteoactivin
Antimicrobial	TSP-1, lactoferrin, CXCL14, Trappin-2, CCL-28, MIG
Anti-inflammatory	IL1-ra, MBL
Embryonic development	DKK1, DKK3
Angiogenesis	VEGF R1, Transferring, TIMP-2
Wound healing	OPN, PAPP-A, FAP

Amniotic fluid preliminary clinical data: Amniotic Fluid recovered and manufactured from the University of Utah's Cell Therapy and Regenerative Medicine program has been clinically used as a Human Cellular and Tissue-Based Product (HCT/P) allograft regulated under 21CFR 1271 in over 2000 applications for over 100 different conditions. A majority of treatments have been for wound and burn healing. Records have been received from over 200 patients receiving AF for the treatment of cervical, thoracic and lumbar radicular pain with no adverse events. Several clinicians throughout the US anecdotally report pain relief for up to one year post-AF injection for their patients. We are, thus, interested in the clinical effects of human amniotic fluid in its ability to reduce and/or eliminate the inflammatory cascade of events associated with radicular lumbar pain.

3. STUDY OBJECTIVES

Determine if epidural injection of amniotic fluid compared to dexamethasone for the treatment of lumbosacral radicular pain due to spinal stenosis results in:

1. Superior safety profile (primary outcome measure).
2. Superior pain relief (primary outcome measure).
3. Superior reduction of functional disability.
4. Superior improvement in psychological functioning.
5. Superior reduction of oral analgesic use, including opioids.
6. Lower incidence of spinal surgery for refractory pain/stenosis symptoms.
7. Superior patient-perceived global impression of change.
8. Need for fewer repeat injections within 3 months from the index injection.
9. Decreased healthcare utilization related to spinal stenosis.

Primary outcomes:

1. The number of adverse events associated with injection (hAF versus dexamethasone) as assessed by a standardized survey and review of systems²⁸.
2. The percentage of participants reporting >50% improvement in numerical pain rating scale (NPRS) at 3 months; back and leg pain separately.

Secondary outcomes:

Difference between groups in:

- 1) Mean change in NPRS pain score (back and leg pain separately).
- 2) The percentage of participants reporting >30% improvement on the Oswestry Disability Index⁶⁴⁻⁶⁶ (ODI) at 3 months.
- 3) Mean change in ODI score improvement.
- 4) The percentage of participants reporting >30% improvement in the Swiss Spinal Stenosis Questionnaire (SSSQ) score.
- 5) Mean change in SSSQ score.
- 6) Mean change in Medication Quantification Scale Version III (MQS III) score. All medications will be entered into the MQS III equation, which is used to determine the standardized cumulative detriment related to a particular medication regimen⁵⁸.
- 7) Mean change in daily morphine equivalent consumption.
- 8) The percentage of participants reporting >30% improvement in PROMIS Global-10 score.
- 9) Mean PROMIS Global-10 score.
- 10) Mean PGIC score.

- 11) Avoidance of spinal surgery (proportion).
- 12) Mean number of repeat injections administered.
- 13) Complications associated with the procedure.
- 14) Additional healthcare utilization related to spinal stenosis symptoms (collected by surveys to quantify all ongoing medical needs including but not limited to physical therapy, occupational therapy, epidural injections, back surgery, additional physician medical visits, additional lumbar spine or other imaging, and any other healthcare utilization related to the participant's spinal stenosis symptoms).

Categorical measures of both pain and function will be used for the primary outcome analysis given recent recommendations by the National Institute of Health⁵⁷.

Rationale/ Research Significance

There is a large population of patients with lumbosacral radicular pain due to spinal stenosis who do not respond to physical therapy or oral medication management, yet wish to avoid spinal surgery or are simply not candidates due to medical co-morbidity. Given the natural history of lumbar spinal stenosis, these patients typically suffer from chronic pain and disability. Currently, the typical treatment for this population is serial corticosteroid injection. The efficacy of the treatment specifically for the indication of spinal stenosis is in question³¹ and is associated with both chronic sequelae²⁹ and the risk, although low, of catastrophic neurologic compromise¹⁴⁻²¹. Alternatively, hAF is a promising new biologic treatment with neuroprotective and regenerative properties. Early studies demonstrate its anti-inflammatory properties, with high levels of anti-inflammatory cytokines, in addition to its ability to assist with the regeneration of peripheral nerves. Furthermore, it has a favorable side-effect profile without concern for long-term sequelae or potential for neurologic compromise. The present study aims to determine if an epidural injection of hAF compared to the corticosteroid dexamethasone is more effective for the treatment of lumbosacral radicular pain due to spinal stenosis, as measured by pain, disability, psychological function, oral analgesic use, and avoidance of surgery. Evidence for the superiority of epidural hAF compared to dexamethasone injection would change the treatment paradigm for refractory radicular pain due to spinal stenosis. Furthermore, even if shown to be non-inferior to epidural dexamethasone, epidural hAF would be favored given its superior safety profile. Thus, this research has the potential to improve outcomes and patient safety in a very large population with chronic pain.

Specific Area of Research: Lumbosacral Radicular Pain due to Spinal Stenosis.

4. DESIGN AND METHODS

Study design

This is a double-blinded, randomized, prospective trial. Participants, study investigators, and research personnel collecting data will remain blinded to participant treatment allocation.

We will obtain local Institutional Review Board (IRB) approval before participant recruitment. A Data Safety Monitoring Plan (DSMP) a-priori stopping rules will be established in collaboration with members of a Data Safety Monitoring Board (DSMB) who will review safety events and evaluate study stopping rules or futility.

Eligibility Criteria

Inclusion Criteria:

1. Participants who are ≥ 18 years old
2. Pain ≥ 4 in unilateral or bilateral leg (with or without back pain). Radicular distribution of leg pain based on history and correlation with advancing imaging. Radicular pain may be fixed or claudicatory in nature. (Score based on Baseline Survey NPRS for leg pain.)
3. Pain resistant to a trial of conservative therapy (i.e., oral steroids, NSAIDs, opioids, muscle relaxants, physical therapy, chiropractic or other non-invasive care) for at least 3 months.
4. Mild-moderate-severe lumbar foraminal or subarticular zone stenosis, and/or mild-moderate central canal spinal stenosis identified by MRI or CT scan according to radiologic criteria (Boden 1996).
5. Ability to read English or Spanish and complete the assessment questionnaires.
6. Must have been 90 days since last lumbar steroid injection.

Exclusion Criteria:

1. Participants in the opinion of the treating investigator who are unwilling or unable to comply with study procedures.
2. Systemic infection or local infection over planned injection site.
3. Bleeding disorder, current use of anticoagulant medications.
4. Intrinsic spinal cord lesions.
5. History of central neurologic, cerebrovascular, demyelinating or muscular disease.
6. Severe vascular, pulmonary or coronary artery disease that limits ambulation including recent myocardial infarction (within the last 6 months).
7. Allergy to medications being used for injection procedures.
8. Women who are pregnant, breastfeeding, or plan to become pregnant while participating in the study. If of child-bearing potential, unwillingness to use effective birth control while participating in the study.
9. Cognitive deficit or motor neuron disease.
10. Spinal instability requiring surgery.
11. History of lumbar spinal fusion surgery
12. Metastatic cancer.
13. Concordant pain with internal rotation of the hip (or known hip joint pathology).

Protocol-specific methods

The study will be conducted at the University of Utah's Orthopaedic Center (UUOC) and the University of Utah South Jordan Health Center (SJHC). With its emphasis on evaluating evidence-based practices and methodological expertise in evaluating and implementing participant-reported outcome measures in the spine, these facilities provide an excellent environment for conducting clinical research. We have a strong portfolio of spine research by a team with broad experience in evaluating the safety of spinal procedures, classifying interventional indications, examining operative and non-operative technologies, and measuring participant-reported outcomes. All participants will undergo a full history and physical examination by a pain medicine subspecialty board-certified physician to ensure accurate diagnosis.

A dedicated clinical study coordinator will be employed to enroll participants. This will include scanning the electronic medical record on a daily basis and face-to-face and email-based communication with the non-operative and operative spine physicians at the University of Utah. The coordinator will be trained in Ethical Conduct for Human Subjects Research, Good Research Practices, HIPAA and computer security. The University of Utah will oversee onboarding through a structured process it has established for regulatory compliance. Given the high clinical volume for this pathology seen at the UUOC and SJHC we envision an abundance of eligible participants.

Adults who are eligible and scheduled to have an epidural injection for lumbosacral radicular pain will be approached by authorized research personnel during their standard-of-care visit. Eligible participants will be consented for the research study and the standard of care epidural injection procedure by study staff. A pre-injection current and average pain score using the Numeric Rating Scale (NPRS) will be recorded. Pain and adjunctive analgesic use will be recorded. An initial disability assessment using the Swiss Spinal Stenosis Questionnaire (SSSQ) will be recorded. Based on history and clinical findings, the attending physician will determine the lumbosacral spinal nerve(s) affected, requiring epidural injection treatment. Additional functional and psychological evaluation will be administered as measured by the Oswestry Disability Index (ODI), Patient Reported Outcomes Measurement Information System (PROMIS) Global-10, Patient Global Impression of Change (PGIC) and Healthcare Utilization Survey.

Participation in the study is voluntary. Participants may choose to not enroll in this research study. Patient care for those who decline to participate in the study will not be impacted and will be presented with the option of epidural injection whether they enroll or decide not to.

Consented participants will be randomized to group #1 or #2 based on a random computer-generated schedule. Permuted blocks ranging in size from 2-12 will be used to achieve balanced treatment groups, carried out through our centralized data collection application. The system is available 24 hours a day, is password-protected and generates an audit trail based on user login. Baseline questionnaires will be ascertained after the consent is signed, but before randomization.

Control measures will be taken to ensure blinded study staff (e.g., treating investigator, research coordinator) and the participant will remain blinded as to which treatment the participant receives.

Group #1: Transforaminal epidural hAF injection at the spinal level indicative of symptoms.

Group #2: Transforaminal epidural dexamethasone injection at the spinal level indicative of symptoms.

Study Enrollment Staggering

To help ensure participant safety and to help limit unreasonable risk the first six participants will be enrolled at a staggered interval. One participant may be enrolled per week for the first six participants. Preliminary safety data for the first six participants will be gathered and reviewed by the Data Safety and Monitoring Board (DSMB). If the DSMB expresses no concern over the preliminary data, the study may proceed with enrollment.

Randomization process.

Eligible and consented participants, including those during the 6-week staggering period, will be randomized using web-enabled content management software designed to support clinical trials. The online system is hosted via a HIPAA-compliant Amazon Web Service server available to credentialed study team members with 2-factor authentication. Randomization assignment will be drawn from randomly permuted blocks ranging in size from 2 to 12, and be stratified by enrolling provider and participants age $\pm$ 65 years. Stratification ensures an equal proportion of cases and controls within each stratum. Randomization to hAF injection or epidural dexamethasone injection will be based on a 1:1 ratio, with each participant having an equal probability of being assigned to either treatment group. Research coordinators will be trained to request a randomization assignment for each participant after completing all required screening, consenting and baseline (pre-treatment) instruments. The online system will not allow ineligible (screen fail) participants to be randomized, and will only allow each participant to be randomized once. Treatment assignment is displayed to the blinded study coordinator and stored on the content management system, along with the participant's study identification, block number, sequence, and stratification data.

Injection Procedures

During the injection procedure, per standard clinical practice, the participant will be positioned prone on a fluoroscopy table. A pre-procedure time-out will be performed, as is standard in the University of Utah spine practice. Once in position for the injection procedure, participants will be randomized to group #1 or #2 based on a random computer-generated schedule. Participants will be blinded to the group to which they are randomized. The physician performing the injection will also be blinded to the participant's group. Unblinded research personnel will prepare the study drug and SOC medication such that the physician and participant cannot distinguish between groups. Identical syringes will be used in both groups, and both dexamethasone and hAF are colorless, translucent, odorless liquids, which are indistinguishable in a syringe. However, unblinded research personnel will prepare the study drug injection and maintain the blind by placing a blank label over the syringe to ensure that the treatment is blinded to the participant and treating physician.

Please note that the following procedure specifications are provided as an example of the current clinical practice, and minor variations in parameters are still considered standard of care and do not constitute protocol deviations: Using fluoroscopic guidance, a lumbosacral epidural injection will be performed. 2-5cc of 1% lidocaine will be injected into the skin and subcutaneous tissue to anesthetize the skin and subcutaneous structures over the site of planned entry to the neural foramen. A 22 or 25 g Whitacre needle (3.5-7") will be used to access the epidural space using the sub-pedicular or infraneural transforaminal approach, depending on individual anatomy at the discretion of the treating physician, consistent with clinical practice guidelines⁵⁴. Needle tip position will be confirmed using anterior-posterior and lateral fluoroscopic views as well as with injection of a standard 0.5-3 mL aliquot of iodinated contrast medium (typically, Omnipaque [Iohexol] but also Isovue [Iopamidol] or other standard commercially available agents, within the standard range of concentrations as per usual care, given frequent iodinated contrast medium shortages) during live fluoroscopy to confirm epidural flow of contrast and to rule out an intravascular injection, the current practice standard. Then, the following exact injection will be performed (study injectate) depending on the previously assigned randomization, 3 mL of hAF or a 3 mL mixture that contains 1 mL of dexamethasone sodium phosphate (1mL, 10 mg/mL)

combined with 2 mL of sterile water will be injected through the spinal needle for unilateral symptoms, for a total injection volume of 3 mL in both groups.

If the participant experiences an acute inflammatory reaction requiring invasive intervention during administration, the administration will be halted and the participant will be treated with appropriate medical intervention.

This study will assess the first six participants with unilateral disease, who will receive unilateral injections as described above. Safety data will be reviewed for the first six participants by the DSMB. If safety criteria are met and the DSMB approves, the study may be opened up for the treatment of participants with bilateral symptoms who will receive bilateral injections. The criteria for assessing safety include:

- Occurrence of an adverse event leading to respiratory failure requiring resuscitation or death that is possibly or probably related to the investigational product.
- One incidence in the hAF group requiring emergent surgical intervention or disseminated infections requiring hospitalization.
- If there is one case of hypersensitivity, defined as an acute inflammatory reaction requiring invasive intervention
- If there is an adverse event that is classified as Severe per protocol that is possibly or probably related to the study drug injection (hAF or steroid)
- At the discretion of the Data Safety Monitoring Board (DSMB). The DSMB will meet regularly to review adverse events, safety analyses, and study data. The DSMB will have access to the actual treatment assignments for each group.

For bilateral symptoms, the injections will be performed on both sides (total of 6 mL in both groups). For multi-level symptoms, the treating physician will choose the one level with most prominent symptoms. Following injection, the needle will be removed from the entry point. Dressing (e.g., bandage) will be placed, and the participant will be transferred to the recovery room. Approximately 15 minutes after the procedure, the participant will be discharged from the clinic with written discharge instructions (current standard practice). A post-procedure pain score will be collected.

Study Drug

Frozen Human Amniotic fluid (hAF) will be provided in a validated container with dry ice by the CellReGen each day a consented participant is seen at the Orthopedic Center or the South Jordan Health Center for the procedure. The hAF will be transferred from the dry ice to the freezer before use, if applicable. If the participant has been randomized to receive the hAF injection, unblinded research staff will prepare the hAF injection and place a blank label over the syringe to ensure blinding. The hAF will be at room temperature before being given to the treating physician to further ensure blinding.

Dexamethasone sodium phosphate will be prepared by unblinded research staff according to institutional best practices. The prepared dexamethasone injection syringe will have a blank label placed over the syringe to ensure blinding. The dexamethasone will be at room temperature before being given to the treating physician to further ensure blinding.

Repeat Injections

If a participant requests an additional injection after the baseline treatment and before Visit 5: 3 Months, they may be offered a repeat injection of the original treatment randomization. The participant and injecting physician will remain blinded to the treatment received. Participants will be limited to 2 injections total (initial injection and 1 repeat) during the first 3 months of the trial. The repeat injection may not occur within 2 weeks of the initial injection, or 3 months after the initial baseline treatment date.

Inclusion Criteria for a Repeat Injection:

- The participant requested it
- The participant has < 50% improvement in pain as measured by NPRS score in *either back or leg pain*
- The participant did not have a Serious Adverse Event (SAE) occurrence that was possibly or probably related to the investigational product
- The Investigator hasn't judged that a repeat injection is not indicated
- The participant did not experience severe hypersensitivity defined as an acute inflammatory reaction requiring invasive intervention during the previous injection

Crossover Injections

If the participant wishes to receive the alternative treatment, they may cross over to the other group at the 3-month time point (± 20 days) after the initial injection but will remain blinded to the original group allocation. Participants who opt to cross over at 3 months will repeat Visit 1, (12-hour phone) call and Visit 2, (48-hour phone call) to assess participant safety and resume the study schedule starting at Visit 6. We believe that this study design will aid with successful recruitment.

Inclusion Criteria for a Cross-Over Injection:

- The participant requested it
- The participant has < 50% improvement in pain as measured by NPRS score in *either back or leg pain*
- The participant did not have a Serious Adverse Event (SAE) occurrence that was possibly or probably related to the investigational product
- The Investigator hasn't judged that a repeat injection is not indicated
- The participant did not experience severe hypersensitivity defined as an acute inflammatory reaction requiring invasive intervention during the previous injection

Repeat Crossover Injections

If a participant had a crossover injection and then requests a repeat injection within 3 months of crossing over, they may be considered for a second cross-over injection. NOTE: Participants cannot receive an additional injection of the original randomization after crossing over. The participant and injecting physician will remain blinded to the treatment received. Participants will be limited to 2 cross-over injections total (initial injection and 1 repeat). Repeat injections may not occur within 2 weeks of or 3 months after the initial crossover treatment date.

Inclusion Criteria for a Repeat Cross-Over Injection:

- The participant requested it
- The participant has < 50% improvement in pain as measured by NPRS score in *either back or leg pain*
- The participant did not have a Serious Adverse Event (SAE) occurrence that was possibly or probably related to the investigational product
- The Investigator hasn't judged that a repeat injection is not indicated
- The participant did not experience severe hypersensitivity defined as an acute inflammatory reaction requiring invasive intervention during the previous injection

Co-interventions

Standard-of-care co-interventions are allowed 3 months after the index study injection. This includes but is not limited to physical therapy, chiropractic care, additional epidural injections, and spinal surgery. All co-interventions will be tallied to assess additional healthcare utilization and cost implications in both groups. We believe that this study design feature will aid with the successful retention of participants.

Study Visit Overview

Visit 1: Screening/Baseline

- Screening & Eligibility
- Informed Consent
- Medical History
- Physical Exam
- Pregnancy test, if applicable
- Concomitant Medications (includes opioid and non-opioid medication usage)
- Randomization
- Study Drug Administration
- Post-Procedure NPRS Score
- Post-Procedure Adverse Events (standardized survey and review of systems²⁸)
- Outcome Measures (NPRS back pain, NPRS leg pain, ODI, SSSQ, PROMIS Global-10)

Healthcare utilization survey (specifically related to spinal stenosis symptoms and included in baseline survey along with other outcome measures.

Visit 1 PM: within 12 hours Post Study Drug Injection

- Follow-up phone call to assess for Adverse Events following Study Drug Injection

Visit 2: 48 hours $\pm$ 5 hours Post Study Drug Injection

- Follow-up phone call to assess for Adverse Events following Study Drug Injection

Visit 3: 3 Weeks $\pm$ 7 days Post Study Drug Injection

- Concomitant Medications (includes any medications used to treat their leg/back pain.)
- Outcome Measures (NPRS back pain, NPRS leg pain, ODI, SSSQ, PROMIS Global-10, PGIC)
- Review of Adverse Events (standardized survey and review of systems²⁸)
- Healthcare utilization survey (Specifically related to spinal stenosis symptoms. Part of the follow-up survey including other outcome measures.)
- 4 weeks (+7) days after the study drug, HLA Class 1 antibody testing will be required for the first 10 enrolled participants
- Repeat Injection* may occur 2 weeks post-Study Drug Injection, includes the following:
 - Post-Procedure NPRS Score
 - Post-Procedure Adverse Events (standardized survey and review of systems²⁸)
 - Pregnancy test, if applicable

*OPTIONAL

Visit 4: 6 Weeks $\pm$ 14 days Post Study Drug Injection

- Concomitant Medications (includes any medications used to treat their leg/back pain.)
- Outcome Measures (NPRS back pain, NPRS leg pain, ODI, SSSQ, PROMIS Global-10, PGIC)
- Review of Adverse Events (standardized survey and review of systems²⁸)
- Healthcare utilization survey (Specifically related to spinal stenosis symptoms. Part of the follow-up survey including other outcome measures.)
- Repeat Injection* may occur 2 weeks post Study Drug Injection, includes the following:
 - Post-Procedure NPRS Score
 - Post-Procedure Adverse Events (standardized survey and review of systems²⁸)
 - Pregnancy test, if applicable

*OPTIONAL

Visit 5: 3 Months $\pm$ 20 days Post Study Drug Injection

- Concomitant Medications (includes any medications used to treat their leg/back pain.)
- Outcome Measures (NPRS back pain, NPRS leg pain, ODI, SSSQ, PROMIS Global-10, PGIC)
- Review of Adverse Events
- Healthcare utilization survey (Specifically related to spinal stenosis symptoms. Part of the follow-up survey including other outcome measures.)
- Cross-Over Injection*, includes the following:
 - Post-Procedure NPRS Score
 - Post-Procedure Adverse Events (standardized survey and review of systems²⁸)
 - Pregnancy Test, if applicable

*OPTIONAL

Visit 6: 6 Months ± 20 days Post Study Drug Injection

- Concomitant Medications (includes any medications used to treat their leg/back pain.)
- Outcome Measures (NPRS back pain, NPRS leg pain, ODI, SSSQ, PROMIS Global-10, PGIC)
- Review of Adverse Events (standardized survey and review of systems²⁸)
- Healthcare utilization survey (Specifically related to spinal stenosis symptoms. Part of the follow-up survey including other outcome measures.)
- Cross-Over Repeat Injection* (may occur 2 weeks post-cross-over treatment and up to 3 months post-cross-over treatment, and may not occur after Visit 6), includes the following:
 - Post-Procedure NPRS Score
 - Post-Procedure Adverse Events (standardized survey and review of systems²⁸)
 - Pregnancy test, if applicable

*OPTIONAL

Visit 7: 12 Months ± 30 days Post Study Drug Injection**

- Concomitant Medications (includes any medications used to treat their leg/back pain.)
- Outcome Measures (NPRS back pain, NPRS leg pain, ODI, SSSQ, PROMIS Global-10, PGIC)
- Review of Adverse Events (standardized survey and review of systems²⁸)
- Healthcare utilization survey (Specifically related to spinal stenosis symptoms. Part of the follow-up survey including other outcome measures.)

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Visit 8: 24 Months± 30 days Post Study Drug Injection**

- Concomitant Medications (includes any medications used to treat their leg/back pain.)
- Outcome Measures (NPRS back pain, NPRS leg pain, ODI, SSSQ, PROMIS Global-10, PGIC)
- Review of Adverse Events (standardized survey and review of systems²⁸)
- Healthcare utilization survey (Specifically related to spinal stenosis symptoms. Part of the follow-up survey including other outcome measures.)

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Outcome assessments

A data collection sheet will be used to record all pre-procedure data:

- 1) Age (years).
- 2) Sex.
- 3) Height (cm).
- 4) Weight (Kg).
- 5) Charlson comorbidity index.
- 6) Duration of pain (weeks).
- 7) Spine surgery history (type, levels)
- 8) Location of lumbosacral radicular pain based on clinical diagnosis: L1, L2, L3, L4, L5, S1.
- 9) Radiologic diagnosis based on MRI of the lumbar spine.
- 10) Typical NPRS score for low back and leg pain, separately.
- 11) ODI score. The ODI is a validated and reliable outcome measure that assesses disability due to low back pain. At least 30% improvement will be used as a robust threshold of clinically meaningful improvement as suggested for functional outcome measures in general.^{59,60}
- 12) SSSQ score. The SSSQ is a validated and reliable outcome measure that assesses pain and disability due to low back and radicular pain due to spinal stenosis.^{61,62} At least 30% improvement will be used as a robust threshold of clinically meaningful improvement as suggested for functional outcome measures in general.^{59,60}
- 13) PROMIS Global-10 score.
- 14) Opioid and non-opioid medication usage. Opioid medications will be converted to morphine equivalents for comparison purposes.

The same data collection sheet will be used to record data immediately following the procedure:

- 1) Adverse events during the procedure.
- 2) NPRS scores for back and leg pain separately.

At intervals of 12 hours, 48 hours, 3 weeks, 6 weeks, 3 months and 6 months, 1 year** and 2 years** we will contact each participant and use a data collection sheet to record all data (data may be obtained at either in-person or remote visits):

- 1) Adverse events attributable to the procedure (a standardized survey and review of systems will be used²⁸).
- 2) NPRS scores for back and leg pain separately.
- 3) ODI score
- 4) SSSQ score
- 5) Opioid and non-opioid medication usage. Opioid medications will be converted to morphine equivalents for comparison purposes. Date medication stopped, if applicable.
- 6) PROMIS Global-10 score.
- 7) PGIC score. Patient global impression of change is a well-established measure of participant-perceived global improvement from a treatment intervention. This measure is often used to establish clinically meaningful changes during validation studies of other outcome measures. At least 30% improvement will be used as a robust threshold of clinically meaningful improvement as suggested for functional outcome measures in general.^{59,60}
- 8) Performance of spinal surgery due to lumbar spinal stenosis symptoms (yes/no, procedure type, date, level(s)).
- 9) Cross-over to the opposite study group (yes/no, date), at Visit 6 only.
- 10) Number of repeat injections (number, AF/dexamethasone), at Visits 6-9.
- 11) Additional healthcare utilization related to spinal stenosis symptoms (collected by surveys to quantify all ongoing medical needs including but not limited to physical therapy, occupational therapy, epidural injections, additional physician medical visits, additional lumbar spine or other imaging, and any other healthcare utilization related to the participant's spinal stenosis symptoms).

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Version: 15 (03MAR2025)

PARTICIPANT STUDY CALENDAR

	Visit 1 Screening /Baseline	Visit 1 PM within 12 hours	Visit 2 48-hour (±5 hours)	Visit 3 3 weeks (± 7 days)	Visit 4 6 Weeks (± 14 days)	Visit 5 3 Months (± 20 days)	Visit 6 6 Months (± 20 days)	Visit 7** 12 Months (± 30 days)	Visit 8** 24 Months (± 30 days)
Eligibility	X								
Informed Consent	X								
¹ Demographics	X								
² Medical History	X								
³ Concomitant Medications	X			X	X	X	X	X	X
Physical Exam	X								
⁴ Pregnancy Test	X								
⁵ Radiologic Imaging	X								
Randomization	X								
⁶ Study Drug Administration	X								
⁷ NPRS score (back and leg, separately)	X			X	X	X	X	X	X
ODI score	X			X	X	X	X	X	X
SSSQ score	X			X	X	X	X	X	X
PROMIS Global-10 score	X			X	X	X	X	X	X
PGIC score				X	X	X	X	X	X
⁷ Adverse Events	X	X	X	X	X	X	X	X	X
Additional Healthcare Utilization	X			X	X	X	X	X	X
⁸ Remote Visit Follow-Up		X	X	X	X	X	X	X	X
⁹ Cross-Over						X			
¹⁰ Laboratory test				X					

¹Demographics include: age, sex, height, weight²Medical History includes: Charlson comorbidity index, spine surgery history, duration of spinal/leg pain³Concomitant Medications include opioid and non-opioid medication usage. Opioid medications will be converted to morphine equivalents for comparison purposes⁴Pregnancy test for women of childbearing potential

⁵Radiologic Imaging (MRI) of the lumbar spine (L1-L5, S1) must be present for clinical diagnosis of spinal stenosis

⁶The initial study drug administration will occur at baseline. The participant may be eligible for a repeat of the baseline injection, at any time from 2 weeks post-baseline and up to 3 months post-baseline. Cross-over may occur at Visit 5 (3 months). If a participant crosses over, they may be eligible for a repeat of the cross-over injection, at any time from 2 weeks post cross-over and up to 3 months post cross-over. No study drug administration may occur after Visit 6 (6 months).

⁷NPRS Score and Adverse Events will be assessed BEFORE and AFTER injections if receiving an injection

⁸Remote Visit Follow-Up will be conducted if repeat injection or cross-over (includes physical exam, pre and post Adverse Events and NPRS score) is not indicated

⁹Study participants may cross over to the other study arm at the 3-month visit, if the participant opts to crossover, then visits 1 (pm call only) and 2 will be repeated. The patient will resume the study schedule at Visit 6. Cross-over is only offered once during the study.

¹⁰HLA Class 1 antibody testing 4 weeks (+7 days) is required for the first 10 enrolled participants

** On February 7, 2025, the Data Safety Monitoring Board recommended ending the study enrollment early. This recommendation was due to the amniotic fluid group demonstrating significantly inferior patient reported outcomes related to pain reduction and a significantly higher rate of repeat injection when compared to the dexamethasone (standard care) group. Notably, the Data Safety Monitoring Board had no safety concerns, and the recommendation was not based on safety differences between the two study groups. As such, enrollment was stopped at 61 participants. 12- and 24-month data will no longer be collected for participants who already surpassed 6 months of follow-up following the Data Safety Monitoring Board's recommendation.

Data collection and coordinating: Study leadership, management and analysis will be overseen by the University of Utah. Data collection will be performed using a web-enabled content management system, STATIX, LLC, that was developed by a Sub-I and is currently used for a PCORI-funded large pragmatic clinical trial of hip and knee joint replacement. The system provides a centralized infrastructure to efficiently manage research participants for multi-site projects, integrating multi-model efforts to collect patient-reported outcomes, including telephone interviewing, online web application, and postage-paid reply mail. Researchers can rapidly develop a follow-up protocol, add or modify survey questions, manage users/sites, and monitor key study performance variables. The password-protected system is hosted on a HIPAA-compliant server that is routinely backed up, ensuring that access to protected information is based on principles of minimum necessity, separation of duties and least privilege, is compliant with Title 21.11, FISMA, and protects privacy.

We estimate baseline questionnaires will take 30 minutes to complete, and follow-up surveys will take 10 minutes. A “window” for collecting each survey will be ± 7 days for the 3-week follow-up, ± 14 days for the 6-week follow-up, ± 20 days for the 3-, and 6-month follow-up, and ± 30 days for the 12 and 24-month assessment. At each window, a minimum of 10 attempts will be made to collect outcomes. Participants will have the option to complete surveys via a postage-paid reply mail survey, telephone interview or a web-enabled survey (accessed using web browsers, or mobile devices).

To facilitate adherence, preference and convenience, we will also allow study participants to complete the follow-up patient-reported outcome surveys using a free mobile device app, available from our data collection vendor, STATIX L.L.C. Key features of the app include seamless integration with our existing data collection service, push notifications to remind participants when it is time to complete a survey, functions to edit contact information and preference, and the ability to complete follow-up surveys through the app with strict adherence to the protocol timing. The mobile app is available as an optional means for collecting the follow-up data; it does not replace the existing means of web-based, telephone interviews and postage-paid reply mail surveys that we use to collect outcomes.

The data collected via the mobile app is stored in the hosted database similar to the other data collection avenues. Once a study participant completes a follow-up survey using the mobile app, no additional follow-up reminders are sent to them. In addition, because the mobile app is simply a formatting platform to deliver our existing web-based surveys onto a mobile screen, the survey questions and answer options are identical to those already used with the other avenues. Changes to contact information, preferences, and PRO survey answers are synchronized between the web-based and the mobile app in real-time.

The mobile app is available for free on both IOS and Android platforms, available from the following sites, which contain screenshots and the app privacy statement.

Apple App Store: <https://apps.apple.com/us/app/statix/id1604450099>

Google Play Store: <https://play.google.com/store/apps/details?id=com.statix>

Participants who opt to use the mobile app will be required to log in using the same credentials (email and password) as they have for the web-based surveys. Participants who use the web or mobile all are asked to create a password at the time that they are first enrolled in the study.

The mobile app will enable push notifications to the end user's device to remind them when a survey is due to be completed. As with any app, participants can disable notifications within their devices. The mobile app will not collect user history, device information, location tracking or other data elements other than the survey data obtained through the app user interface as part of the research study protocol.

Data storage

All collected data will be stored in a password-protected computer located in the University of Utah Orthopaedic Center. Participants will each be assigned an ID number, which will be used as the sole identifier on any documents. Participant data will be compiled into a single password-protected file in which participants will only be identified by their ID number. An enrollment log will be the only file where participant names are correlated with ID numbers. This will be kept in a separate, secure, password-protected file in the University of Utah Orthopaedic Center.

Sample size and justification

Our original power analysis called for study groups sizes of 56; 112 total participants. The necessary sample size was determined based on a review of past literature and power analysis. Power analysis was performed based on the primary outcome measure of the proportion of participants who experienced >50% pain reduction. The highest quality study to date demonstrated a successful response of 38% when using dexamethasone epidural injection for the indication of leg pain due to spinal stenosis. While there are no studies of epidural hAF injection, response rates from pilot data range from 70-75%, so a conservative estimate of 65% was used. Using these parameters, 50 participants in each group would provide 80% power to detect a significant difference between groups using an alpha value of 0.05 (Pocock 1983).⁵⁶ We had planned to enroll a total of 112 participants to account for an estimated attrition rate of 10% which will still allow for adequate power to detect a statistically significant difference at a total sample size of 100 participants.

However, the Data Safety Monitoring Board recommended ending the study enrollment early. This recommendation was due to the amniotic fluid group demonstrating significantly inferior patient reported outcomes related to pain reduction and a significantly higher rate of repeat injection when compared to the dexamethasone (standard care) group. Notably, the Data Safety Monitoring Board had no safety concerns, and the recommendation was not based on safety differences between the two study groups. As such, enrollment was stopped at 61 participants.

Data Analysis

Analytic approach: Intention to treat analyses for primary effectiveness analysis will be used; this evaluates outcomes based on the treatment arm to which participants were originally assigned.

Long-term outcomes past 3 months will be evaluated using both intention-to-treat and as-treated analysis given cross-over and drop-out.

Primary outcomes:

1. The number of adverse events associated with injection (hAF versus dexamethasone) as assessed by a standardized survey and review of systems²⁸.
2. The percentage of participants reporting >50% improvement in NPRS at 3 months; back and leg pain separately.

Secondary outcomes:

Difference between groups in:

- 1) Mean change in NPRS pain score (back and leg pain separately).
- 2) The percentage of participants reporting >30% improvement on the ODI at 3-months.
- 3) Mean change in ODI score improvement.
- 4) The percentage of participants reporting >30% improvement in the SSSQ score.
- 5) Mean change in SSSQ score.
- 6) Mean change in MQS III score. All medications will be entered into the medication quantification Scale III equation, which is used to determine the standardized cumulative detriment related to a particular medication regimen⁵⁸.
- 7) Mean change in daily morphine equivalent consumption.
- 8) The percentage of participants reporting >30% improvement in PROMIS Global-10 score.
- 9) Mean PROMIS Global-10 score.
- 10) Mean PGIC score.
- 11) Avoidance of spinal surgery (proportion).
- 12) Mean number of repeat injections administered.
- 13) Complications associated with the procedure.
- 14) Additional healthcare utilization related to spinal stenosis symptoms (collected by survey to quantify all ongoing medical needs including but not limited to physical therapy, occupational therapy, epidural injections, additional physician medical visits, additional lumbar spine or other imaging, and any other healthcare utilization related to the participant's spinal stenosis symptoms).

Categorical measures of both pain and function will be used for the primary outcome analysis given recent recommendations by the National Institute of Health⁵⁷.

Subgroup analysis:

- 1) Stratification of pain (NPRS) and functional (ODI) outcomes by demographic factors in order to determine positive prognostic factors.

Appropriate models (e.g., normal, Poisson, gamma, binomial) will be fit for secondary patient-reported outcomes, assessment of safety events, and to explore heterogeneous treatment effects, but using conventional hypothesis testing with a two-sided alpha of 0.05. For these secondary analyses, we will compare rates of safety, pain response and other patient-reported outcomes in subgroups defined by baseline level of pain and function, age (grouped by decade), duration of back pain symptoms, body mass index (BMI), and Charlson comorbidity index. We will analyze dichotomous safety outcomes, such as emergency room visits, using a discrete-time survival analysis given that we plan to collect safety data at regular intervals. Specifically, we will generate pseudo-observation event indicators for each time point, and fit a generalized model for a binomial outcome with a complementary log-log link.

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Statistical analysis

Data will first be checked for distributional form and outliers using summary statistics and graphical displays. Data will be analyzed using SPSS version 22 (IBM 2013) or STATA-MP-16 (College Station, TX). The level of significance will be set at 0.05. Two-sided testing will be used for all hypothesis testing.

To illustrate the demographic, radiologic, and procedural characteristics of the study sample, means and standard deviations or medians and interquartile differences (depending on the distribution of data) will be calculated for continuous variables. Demographic, clinical, and radiologic differences will be compared between the groups to confirm successful randomization.

In comparing groups with regard to categorical variables, Chi Square or Fisher's exact tests will be used. Proportions and 95% Confidence Intervals will also be calculated to confirm that any differences between groups are truly distinguished. Depending on the distribution of continuous data, Student's T-tests or Mann-Whitney U tests will be used to compare means or medians, respectively.

Multiple regression analysis will be used to determine if any demographic or clinical variables (age, BMI, duration of pain, etc.) are independently associated with successful pain (NPRS) and functional (SSSQ) outcomes.

Longitudinal models of outcome using a time-varying covariate indicating when treatment occurred will be used to account for changes in outcome related to group cross-over.

Study Monitoring

Site monitoring visits will be performed by a trained site monitor during the study period to ensure regulatory compliance, participant safety and to monitor the quality of data collected. Essential document binders, regulatory documents and data collection forms may be reviewed. Interim visits will take place depending on budget, site enrollment, and compliance issues identified. The site monitor will provide each site with a written report, and sites will be required to follow up on any deficiencies. It is anticipated that the study monitoring visits for this protocol will consist of a site initiation visit (before participant enrollment), interim visits, and a close-out visit. The site initiation may take place as group training made up of site investigators and research assistants.

Pharmacy Monitoring

The Cell Therapy and Regenerative Medicine (CellReGen) must maintain adequate records of all dispensed study drug. The CellReGen may be monitored and requested to send copies of these documents.

Record Access

The medical record and study files (including informed consent documents) must be made available to the site monitor, upon request, for source verification of study documentation. In addition, medical information and data generated by this study must be available for inspection upon request by representatives (when applicable) of the Food and Drug Administration (FDA), other Federal funders or study sponsors, and the Institutional Review Board (IRB).

Protection of Human Participants

Institutional Review Board (IRB) Approval

IRB approval must be obtained prior to participating in the study. The site monitor will track IRB approval status and will not permit participant enrollment without documentation of initial IRB approval and maintenance of that approval throughout subsequent years of the project.

Informed Consent

For all participants ≥ 18 years of age, informed consent is required. Participants who are capable of giving consent and who are alert and competent will be asked, following an appropriate discussion of risks and benefits, to give consent to the study. For those with diminished mental capacity, a Legal Authorized Representative will be used.

Risks/Benefits

Risks of study participation are the same as those for any standard fluoroscopically guided lumbosacral epidural injection frequently performed at the University of Utah Orthopaedic Center, University of Utah South Jordan Health Center, and University of Utah Farmington Health Center. These risks will be explained to the participants in the trial as part of their standard consent for the injection procedure separately. These include local infection, epidural hematoma or abscess, dural puncture and potential post-dural puncture headache, paresthesia during needle placement, pain at the injection site, failure of technique and an allergy to latex or medications being used.

Utilizing fluoroscopy, the risk of nerve damage, spinal cord injury or intravascular injection is less than 1:5,000,000. The recovery is no longer than that of a standard epidural injection performed in the University of Utah Health Clinics at present.

The benefits of active drug therapy in this population are unknown; however, it is postulated that there may be a potential benefit in pain and functional outcomes for patients with radicular pain due to spinal stenosis.

Data and Safety Monitoring Plan

Data Safety Monitoring Board (DSMB)

The study will have a Data Safety Monitoring Board (DSMB). The DSMB will have a charter, will approve the protocol before implementation, and will review interim analyses as applicable. The purpose of the DSMB is to advise the sponsors and Principal Investigator(s) regarding the continuing safety of study participants and the continuing validity and scientific merit of the study. The DSMB is responsible for monitoring the accrual of study participants, adherence to the study protocol, assessments of data quality, performance of the individual Clinical Center, review of serious adverse events and other participant safety issues, and review of formal interim statistical analyses of treatment efficacy.

Adverse Event Reporting

The site investigator is responsible for evaluating all adverse events at their clinical center and ensuring they are properly reported. The nature of each experience, date and time (where appropriate) of onset, outcome, course, and relationship to treatment will be documented.

Definition of Adverse Event and Serious Adverse Event

Adverse Event (AE) means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR (a)). An event constitutes a disease, a set of related signs or symptoms, or a single sign or symptom.

Note: An increase in a participant's NPRS scores immediately following the study treatment will not be captured as an adverse event as this is a common and expected side effect of epidural injection procedures.

Serious Adverse Event (SAE): A serious adverse event (SAE) for this population is an adverse event that:

- Results in death; or
- Is life-threatening (the participant was, in the view of the site investigator, in immediate danger of death from the event as it occurred); or
- Requires inpatient hospitalization or prolongs an existing hospitalization; or
- Results in persistent or significant disability or incapacity; or
- Results in a congenital anomaly/birth defect; or
- Any other event that, based upon appropriate medical judgment, may jeopardize the participant's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

Classification of an Adverse Event (Relatedness, Severity, and Expectedness)

Relatedness: The suspected relationship between study interventions and any adverse event will be determined by the site investigator using the criteria below. Relatedness must be assessed by an investigator and may not be assessed by a research coordinator.

Not Related: The event is clearly related to other factors, such as the participant's clinical state, therapeutic interventions, or concomitant drugs administered to the participant.

Possibly Related: The event follows a compatible temporal sequence from the time of beginning the assigned study intervention but could have been produced by other factors such as the participant's clinical state, therapeutic interventions, or concomitant drugs administered to the participant.

Probably Related: The event follows a reasonable temporal sequence from the time of beginning the assigned study intervention, and cannot be reasonably explained by other factors such as the participant's clinical state, therapeutic interventions, or concomitant drugs administered to the participant.

Severity: The severity, which is a measure of intensity, of clinical adverse events and laboratory abnormalities will be recorded by the site investigator and categorized. The following guidelines will be used to describe severity.

Mild: The event requires minimal or no treatment and does not interfere with the participant's daily activities.

Moderate: The event results in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.

Severe: The event interrupts a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".

Expectedness of the Event: All adverse events, including serious adverse events, will be evaluated as to whether their occurrence was expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information described for the study intervention.

Expected: An event is considered expected if it is known to be associated with the underlying condition or is related to the study intervention and is mentioned in the protocol, informed consent, or other study documents. An event may be expected despite the study participant's clinical state immediately prior to the event.

Expected adverse events for this study are:

- Pain at the injection site
- Soreness/temporary pain flare (of typical back and leg pain)
- Temporary inflammation at the injection site
- Temporary Headache, facial flushing, palpitations
- Mild, temporary sleeplessness
- Infection
- Minor bleeding

Unexpected: An event is considered unexpected if there is no prior data linking this event with either the condition or intervention under study or an event that occurred unexpectedly in the course of treatment.

Treatment or Action Taken: For each adverse event, the site investigator will record whether an intervention was required:

- Medical or surgical procedure
- Concomitant medication: started, changed, or discontinued
- Other, specify
- No action taken

Outcome of Event: Finally, the site investigator will record the clinical outcome of each adverse event as follows:

- Death
- Recovered and the participant returned to baseline status
- Recovered with permanent sequelae
- Symptoms persist

Time Period for Adverse Events

For purposes of this study, events that occur following randomization through the last follow-up visit at 24 months will be reported as adverse events. Serious adverse events, unexpected medically attended events, and new onset chronic illnesses will be recorded from randomization through twenty-four months after the last study dose. Specifically, events that occur following informed consent to participate in the study, but before actual randomization, are not adverse events. These should be recorded as baseline conditions.

Data Collection Procedures for Adverse Events

After participant randomization, all adverse events (including serious adverse events) will be recorded according to relatedness, severity, and expectedness, as well as their duration and any treatment prescribed. Any medical condition present at the time of randomization, recorded in the participant's baseline history at study entry, which remains unchanged or improves (unless the clinician feels it is clinically relevant), will not be recorded as an adverse event at subsequent evaluations. However, the worsening of a medical condition that was present at the time of randomization will be considered a new adverse event and reported.

Adverse events will be coded using the MedDRA coding vocabulary. Coding will be done centrally at the Data Coordinating Center as this requires specific training.

Unanticipated Problems (UP)

Unanticipated problems (UP) are defined as incidents, experiences, or outcomes that are unexpected, related or possibly related to participation in the study, and suggest that the research places participants at a greater risk of harm than was previously known or recognized. The site investigator is responsible for ensuring unanticipated problems are reported to the DSMB within 24 hours of becoming aware of the event. A detailed completed report will be required to be sent to the DSMB in an expedited manner (as close to 24 hours as possible). In accordance with local IRB requirements, the site investigator may be required to report such unanticipated problems to the IRB and FDA in addition to notifying the DSMB. If the medical monitor believes that such an event warrants emergent suspension of enrollment in the trial, the DSMB will notify the study investigator (Dr. McCormick) to cease enrollment in the trial. Resumption of enrollment will not occur without the consent of the DSMB and the FDA.

Monitoring Serious Adverse Events

A qualified physician will be designated to fulfill the function of the medical monitor for this study. Site investigators and/or research coordinators will report serious adverse events to the DSMB within 24 hours of becoming aware of the event. A detailed completed report will be required to be sent to the DSMB within 3-working days of the event, and the medical monitor will assess all serious adverse events reported by site investigators.

For each of these serious adverse events, the site investigator will provide sufficient medical history and clinical details for a safety assessment to be made regarding the continuation of the trial. The medical monitor will sign off on each SAE report after review. All SAE reports will be retained at the DSMB, and all SAE reports will be available for review by DSMB members. The SAE reporting process may be incorporated into the Electronic Data Capture (EDC) System in use for the study.

In the unlikely event that the medical monitor believes an unexpected and study-related SAE warrants emergent cessation of enrollment in the trial, CellReGen staff and the DSMB chairperson will be immediately consulted. If these individuals concur with the judgement of the medical monitor, or if the CellReGen staff and the DSMB chairperson cannot be reached expeditiously, the DSMB will notify the study investigator (Dr. McCormick) and all site investigators to cease enrollment in the trial. Resumption of enrollment will not occur without consent of the DSMB and the FDA.

In accordance with local IRB requirements, the site investigator may be required to report such events to the IRB in addition to notifying the DSMB. After notification of the CellReGen, and the DSMB chairperson, of serious, unexpected, and study-related adverse events or unanticipated problems (UP), decisions will be made on whether to continue the study without change, and whether to convene the entire DSMB for an emergent meeting. If a decision is made to suspend enrollment in the trial, this will be reported to the study investigator (Dr. McCormick), who will be instructed to report this to their local IRB.

The DSMB will review all adverse events (not necessarily serious, unexpected, and study-related) during scheduled DSMB meetings. The DSMB will prepare a Summary Report of Adverse Events, classified with the MedDRA coding system.

During these routine meetings of the DSMB, they will review adverse events, safety analyses, and study data. The DSMB can use its discretion on suspending enrollment in the trial due to reported adverse events, safety analyses and findings from the study data. The DSMB will be un-blinded to the treatment arm.

Follow-up of Serious, Unexpected and Related Adverse Events:

All serious, unexpected and related adverse events that are unresolved at the time of the participant's termination from the study will be followed by the Clinical Center investigators until the events are resolved, the participant is lost to follow-up, the adverse event is otherwise explained or has stabilized, or 24-months have passed from the time of last study dose in participants who reached the 24-month follow-up milestone prior to the time that the Data Safety Monitoring Board recommended ending enrollment early or 3-months have passed from the time of the last study dose in participants who were still within 3-months of the last study dose at the time that the Data Safety Monitoring Board recommended ending enrollment early.

Participant Withdrawal from the Study

A participant may withdraw from the study at any time, without a penalty or loss of standard of care. Data collected up until the point of withdrawal will still be used in efficacy and safety analyses.

Administration of a Repeat, Cross-Over or Repeat Crossover Injection

Inclusion Criteria for Additional Injections:

- The participant requested it
- Participants have < 50% improvement in pain as measured by NPRS score in *either* back *or* leg pain
- The participant did not have a Serious Adverse Event (SAE) occurrence that was possibly or probably related to the investigational product
- The Investigator hasn't judged that a repeat injection is not indicated
- The participant did not experience severe hypersensitivity defined as an acute inflammatory reaction requiring invasive intervention during the previous injection

Discontinuation of the Study

The study will be discontinued if:

- Any participant has an adverse event leading to respiratory failure requiring resuscitation or death that is possibly or probably related to the investigational product.
- Two or more incidences in the hAF group requiring emergent surgical intervention or disseminated infections requiring hospitalization.
- If there is one case of hypersensitivity, defined as an acute inflammatory reaction requiring invasive intervention
- If there an adverse event that is classified as Severe per protocol that is possibly or probably related to the study drug injection (hAF or steroid)
- At the discretion of the Data Safety Monitoring Board (DSMB). The DSMB will meet regularly to review adverse events, safety analyses, and study data. The DSMB will have access to the actual treatment assignments for each group.

In the event study enrollment has been suspended due to the above criteria or any other reason the FDA, the DSMB and the Sponsor must be notified. Enrollment and treatment will be resumed after a review of the incident and of any corrective and preventive actions have been established with consultation between the study team, the Sponsor, and the DMSB. The study may not resume without FDA approval.

STUDY TRAINING

A formal training program for investigators and research staff will be held prior to the start of enrollment. The training program will cover regulatory topics and Good Clinical Practice. The training will also provide in-depth explanations regarding study procedures, clinical care, adverse event reporting, data entry procedures, quality assurance, site monitoring, and the informed consent process. A manual of operations will be provided to each investigator prior to the start of enrollment. The manual will detail specific information about the study procedures, regulatory information, safety reporting, and other necessary information. Updates and revisions to the manual will be made available electronically.

REGULATORY CONSIDERATIONS

Food and Drug Administration

This trial is being conducted under an Investigational New Drug application approved by the Food and Drug Administration (Investigational New Drug application #26950). The clinical investigator at each participating site will complete a Form FDA 1572, "Statement of Investigator."

*FDA Annual reporting will be submitted within 60 days of the anniversary of the date that the IND went into effect. (21 CFR 212.33)

Health Insurance Portability and Accountability Act

Data elements collected may include the date of birth and date of admission. Prior to statistical analyses, dates will be used to calculate participant age at the time of the study events. The final data sets (used for study analyses and archived at the end of the study) will be de-identified, and will exclude these specific dates.

Inclusion of Women and Minorities

There will be no exclusion of participants based on gender, race, or ethnicity.

ClinicalTrials.gov Requirements

This study is registered on clinicaltrials.gov. NCT04537026.

Retention of Records

For federally funded studies subject to the Common Rule, records relating to the research conducted shall be retained for at least 3-years after completion of the research. Completion of the research for this protocol should be anticipated to include planned primary and secondary analyses, as well as subsequent derivative analyses. Completion of the research also entails completion of all publications relating to the research. All records shall be accessible for inspection and copying by authorized representatives of the regulatory authorities at reasonable times and in a reasonable manner [45 CFR §46.115(b)].

Amendments

Any amendments or administrative changes to an IRB-approved protocol will not be initiated without the submission of an amendment for IRB review and approval. Any amendments to the protocol that significantly affect the safety of participants, the scope of the investigation, or the scientific quality of the study are required to submit the amendment for FDA review.

Annual Reports

An annual progress report will be submitted to the FDA within 60 days of the anniversary of the date that the IND went into effect. (21 CFR 312.33)

References:

1. Melancia JL, Francisco AF, Antunes JL. Spinal stenosis. In: Handbook of clinical neurology. Elsevier; 2014. p. 541–9.
2. Babu AN, McCormick Z, Kennedy DJ, Press J. Local, national, and service component cost variations in the management of low back pain: Considerations for the clinician. J Back Musculoskelet Rehabil. 2016;
3. Daffner SD, Wang JC. The pathophysiology and nonsurgical treatment of lumbar spinal stenosis. Instr Course Lect. 2009;

4. Englund J. Lumbar spinal stenosis. *Curr Sports Med Rep*. 2007;6(1):50–5.
5. Ammendolia C, Stuber KJ, Rok E, Rampersaud R, Kennedy CA, Pennick V, et al. Nonoperative treatment for lumbar spinal stenosis with neurogenic claudication. *Cochrane Database of Systematic Reviews*. 2013.
6. Macedo LG, Hum A, Kuleba L, Mo J, Truong L, Yeung M, et al. Physical Therapy Interventions for Degenerative Lumbar Spinal Stenosis: A Systematic Review. *Phys Ther*. 2013;
7. Weinstein JN, Tosteson TD, Lurie JD, Tosteson ANA, Blood E, Hanscom B, et al. Surgical versus nonsurgical therapy for lumbar spinal stenosis. *N Engl J Med*. 2008;
8. Overdevest GM, Jacobs W, Vleggeert-Lankamp C, Thomé C, Gunzburg R, Peul W. Effectiveness of posterior decompression techniques compared with conventional laminectomy for lumbar stenosis. *Cochrane Database of Systematic Reviews*. 2015.
9. Friedly J, Chan L, Deyo R. Increases in lumbosacral injections in the medicare population: 1994 to 2001. *Spine (Phila Pa 1976)*. 2007;
10. Manchikanti L, Pampati V, Falco FJE, Hirsch JA. Assessment of the growth of epidural injections in the medicare population from 2000 to 2011. *Pain Physician*. 2013.
11. El-Yahchouchi C, Plastaras C, Maus T, Carr C, McCormick Z, Geske J, et al. Complication rates of transforaminal and interlaminar epidural steroid injections: A multi-institutional study. *Pain Med*. 2014;15(8).
12. Carr C, Plastaras C, Pingree M, Smuck M, Maus T, Geske J, et al. Adverse event rates in interventional spine procedures: A multi-institutional study. *Pain Med*. 2014;15(8).
13. Kennedy DJ, Levin J, Rosenquist R, Singh V, Smith C, Stojanovic MP, et al. Epidural Steroid Injections are Safe and Effective: Multisociety Letter in Support of the Safety and Effectiveness of Epidural Steroid Injections. *Pain Medicine (United States)*. 2015.
14. Muro K, O'Shaughnessy B, Ganju A. Infarction of the cervical spinal cord following multilevel transforaminal epidural steroid injection: Case report and review of the literature. *J Spinal Cord Med*. 2007;
15. Kennedy DJ, Dreyfuss P, Aprill CN, Bogduk N. Paraplegia following image-guided transforaminal lumbar spine epidural steroid injection: Two case reports. *Pain Med*. 2009;
16. Brouwers PJAM, Kottink EJBL, Simon MAM, Prevo RL. A cervical anterior spinal artery syndrome after diagnostic blockade of the right C6-nerve root. *Pain*. 2001;

17. Quintero N, Laffont I, Bouhmidi L, Rech C, Schneider AE, Gavardin T, et al. Transforaminal epidural steroid injection and paraplegia: case report and bibliographic review. In: *Annales de readaptation et de medecine physique: revue scientifique de la Societe francaise de reeducation fonctionnelle de readaptation et de medecine physique*. 2006. p. 242–7.
18. Houten JK, Errico TJ. Paraplegia after lumbosacral nerve root block: Report of three cases. *Spine J*. 2002;
19. Huntoon MA, Martin DP. Paralysis after transforaminal epidural injection and previous spinal surgery. *Reg Anesth Pain Med*. 2004;
20. Somayaji HS, Saifuddin A, Casey AT, Briggs TW. Spinal cord infarction following therapeutic computed tomography-guided left L2 nerve root injection. *Spine (Phila Pa 1976)*. 2005;
21. Glaser SE, Falco F. Paraplegia following a thoracolumbar transforaminal epidural steroid injection. *Pain Physician*. 2005;
22. Benzon HT, Chew TL, McCarthy RJ, Benzon HA, Walega DR. Comparison of the particle sizes of different steroids and the effect of dilution: A review of the relative neurotoxicities of the steroids. *Anesthesiology*. 2007;
23. Derby R, Lee SH, Date ES, Lee JH, Lee CH. Size and aggregation of corticosteroids used for epidural injections. *Pain Med*. 2008;
24. Nahm FS, Lee CJ, Lee SH, Kim TH, Sim WS, Cho HS, et al. Risk of intravascular injection in transforaminal epidural injections. *Anaesthesia*. 2010;
25. Baker R, Dreyfuss P, Mercer S, Bogduk N. Cervical transforaminal injection of corticosteroids into a radicular artery: A possible mechanism for spinal cord injury. *Pain*. 2003;
26. Lu J, Ebraheim NA, Biyani A, Brown JA, Yeasting RA. Vulnerability of great medullary artery. In: *Spine*. 1996.
27. Kroszczynski AC, Kohan K, Kurowski M, Olson TR, Downie SA. Intraforaminal location of thoracolumbar anterior medullary arteries. *Pain Med (United States)*. 2013;
28. Plastaras C, McCormick Z, Garvan C, Macron D, Joshi A, Chimes G, et al. Fluoroscopically Guided Lumbosacral Transforaminal Epidural Steroid Injections: Adverse Events and Predictive Factors. 2015;
29. Nesbitt LT. Minimizing complications from systemic glucocorticosteroid use. *Dermatologic Clinics*. 1995.

30. Ghahreman A, Ferch R, Bogduk N. The efficacy of transforaminal injection of steroids for the treatment of lumbar radicular pain. *Pain Med (United States)*. 2010;
31. Friedly JL, Comstock BA, Turner JA, Heagerty PJ, Deyo RA, Sullivan SD, et al. A randomized trial of epidural glucocorticoid injections for spinal stenosis. *N Engl J Med*. 2014;
32. Fukusaki M, Kobayashi I, Hara T, Sumikawa K. Symptoms of spinal stenosis. Do not improve after epidural steroid injection. *Clin J Pain*. 1998;
33. Manchikanti L, Cash KA, McManus CD, Pampati V, Abdi S. Preliminary results of a randomized, equivalence trial of fluoroscopic caudal epidural injections in managing chronic low back pain: Part 4 - Spinal stenosis. *Pain Physician*. 2008;
34. Smith CC, McCormick ZL, Mattie R, MacVicar J, Duszynski B, Stojanovic MP. The effectiveness of lumbar transforaminal injection of steroid for the treatment of radicular pain: a comprehensive review of the published data. *Pain Med*. 2019;
35. Chiu CC, Chuang TY, Chang KH, Wu CH, Lin PW, Hsu WY. The probability of spontaneous regression of lumbar herniated disc: A systematic review. *Clinical Rehabilitation*. 2015.
36. Kobayashi S. Pathophysiology, diagnosis and treatment of intermittent claudication in patients with lumbar canal stenosis. *World J Orthop*. 2014;
37. Underwood MA, Gilbert WM, Sherman MP. Amniotic fluid: not just fetal urine anymore.(State of the Art)(Report). *J Perinatol*. 2005;
38. Prusa AR, Marton E, Rosner M, Bernaschek G, Hengstschläger M. Oct-4-expressing cells in human amniotic fluid: A new source for stem cell research? *Hum Reprod*. 2003;
39. Bottai D, Cigognini D, Nicora E, Moro M, Grimoldi MG, Adami R, et al. Third trimester amniotic fluid cells with the capacity to develop neural phenotypes and with heterogeneity among sub-populations. *Restor Neurol Neurosci*. 2012;
40. Shimberg M. The use of amniotic-fluid concentrate in orthopaedic conditions. *JBJS*. 1938;20(1):167–77.
41. Johnson HL. Peritoneal immunization. *Am J Surg*. 1936;34(2):266–71.
42. Ojo VA, Okpere EE, Obaseiki-Ebor EE. Antimicrobial properties of amniotic fluid from some Nigerian women. *Int J Gynecol Obstet*. 1986;24(2):97–101.
43. Ismail MA, Salti GI, Moawad AH. Effect of amniotic fluid on bacterial recovery and growth: Clinical implications. *Obstetrical and Gynecological Survey*. 1989.

44. Özgenel GY, Filiz G, Özcan M. Effects of human amniotic fluid on cartilage regeneration from free perichondrial grafts in rabbits. *Br J Plast Surg*. 2004;
45. Özgenel GY, Filiz G. Effects of human amniotic fluid on peripheral nerve scarring and regeneration in rats. *J Neurosurg*. 2003;
46. Karaçal N, Koşucu P, Çobanglı Ü, Kutlu N. Effect of human amniotic fluid on bone healing. *J Surg Res*. 2005;
47. Castro-Combs J, Noguera G, Cano M, Yew M, Gehlbach PL, Palmer J, et al. Corneal wound healing is modulated by topical application of amniotic fluid in an ex vivo organ culture model. *Exp Eye Res*. 2008;
48. Nyman E, Huss F, Nyman T, Junker J, Kratz G. Hyaluronic acid, an important factor in the wound healing properties of amniotic fluid: In vitro studies of re-epithelialisation in human skin wounds. *J Plast Surg Hand Surg*. 2013;
49. Weissenbacher T, Laubender RP, Witkin SS, Gingelmaier A, Schiessl B, Kainer F, et al. Influence of maternal age, gestational age and fetal gender on expression of immune mediators in amniotic fluid. *BMC Res Notes*. 2012;
50. Merimee TJ, Grant M, Tyson JE. Insulin-like growth factors in amniotic fluid. *J Clin Endocrinol Metab*. 1984;59(4):752–5.
51. Adzick NS, Lorenz HP. Cells, matrix, growth factors, and the surgeon: The biology of scarless fetal wound repair. *Annals of Surgery*. 1994.
52. Gao X, Devoe LD, Given KS, Mustoe TA. Effects of amniotic fluid on proteases: A possible role of amniotic fluid in fetal wound healing. *Ann Plast Surg*. 1994;
53. Pierce J, Jacobson P, Benedetti E, Peterson E, Phibbs J, Preslar A, et al. Collection and characterization of amniotic fluid from scheduled C-section deliveries. *Cell Tissue Bank*. 2016;17(3):413–25.
54. Bogduk N. Practice guidelines for spinal diagnostic and treatment procedures. International Spine Intervention Society. International Spine Intervention Society; 2013.
55. El-Yahouchi C, Geske JR, Carter RE, Diehn FE, Wald JT, Murthy NS, et al. The noninferiority of the nonparticulate steroid dexamethasone vs the particulate steroids betamethasone and triamcinolone in lumbar transforaminal epidural steroid injections. *Pain Med (United States)*. 2013;
56. Seldrup J, Pocock SJ. *Clinical Trials. A Practical Approach*. Stat. 1985;

57. Deyo RA, Dworkin SF, Amtmann D, Andersson G, Borenstein D, Carragee E, et al. Report of the NIH task force on research standards for chronic low back pain. *Pain Med* (United States). 2014;
58. Gallizzi M, Gagnon C, Harden RN, Stanos S, Khan A. Medication quantification scale Version III: Internal validation of detriment weight using a chronic pain population. *Pain Pract*. 2008;
59. Ostelo RW, Deyo RA, Stratford P, Waddell G, Croft P, Von Korf M, et al. Interpreting change scores for pain and functional status in low back pain: towards international consensus regarding minimal important change. *Spine (Phila Pa 1976)* 2008;33(1):90–4.
60. Asher AM, Oleisky ER, Pennings JS, Khan I, Sivaganesan A, Devin CJ, Bydon M, Asher AL, Archer KR. Measuring clinically relevant improvement after lumbar spine surgery: is it time for something new? *Spine J*. 2020 Jun;20(6):847-856.
61. Comer CM, Conaghan PG, Tennant A. Internal construct validity of the Swiss Spine Stenosis Questionnaire: Rasch analysis of a disease-specific outcome measure for lumbar spinal stenosis. *Spine (Phila Pa 1976)*. 2011 Feb 1
62. Stucki G, Daltroy L, Liang MH, et al. Measurement properties of a self-administered outcome measure in lumbar spinal stenosis. *Spine* 1996; 21: 796–803.
63. Radloff LS. The CES-D scale: A self-report depression scale for research in the general population. *Applied Psychological Measurement*. 1977;1: 385–401
64. Fairbank JC, Pynsent PB, *The Oswestry Disability Index*. *Spine (Phila Pa 1976)*, 2000. **25** (22): p. 2940-52; discussion 2952. PMID: 11074683
65. Fritz JM, Irrgang JJ, *A comparison of a modified Oswestry Low Back Pain Disability Questionnaire and the Quebec Back Pain Disability Scale*. *Phys Ther*, 2001. **81**(2): p. 776-88. PMID: 11175676
66. Asher, A. M., et al. (2020). "Measuring clinically relevant improvement after lumbar spine surgery: is it time for something new?" *Spine J* **20**(6): 847-856.